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DASPE 20 $\mu\text{g}/\text{ml}$

Plak TCE- ket voor H β

TCE-amp 2x

TCE- kanaal 2x

kij maken 1. Strategiem Plak SP

2. SE

3. LP

4. LE

Volgen post 30j ; u XC-CP

1435 H β by 10 μ

1435 K17 hby

10

1 } $\pm 25 \rightarrow 2,5 \cdot 10^{12}/\text{ml}$

2

3

4

gebruikt voor opgevoerde h β by

[redacted] library 1 1/2 g agarose : stock + 340 C
 [redacted] PEG prep
 [redacted] lib. 8-14 agarose

Control : nr2, nr5
 V₁ 1/4, V₂ 7/8

10¹³/ml = 10¹²/100 μl
 OK
 10 + 990 = 10¹⁰ / 100 μl
 10 + 990 = 10⁹
 10 + 990 = 10⁸ 5 · 10⁵
 10 + 990 = 10⁷ 5 · 10³
 10 + 990 = 10⁶ } interpolate 50
 10 + 990 = 10⁵ } 0.5

14/7 PEG tot 100
 100 - 100 = 0

phabs - MPBS : 4/3 x 20% = 27% = 1.35 g / 50 ml
 4.0% = 2 g / 50 ml

pre incubate : 12 x 42 μl phabs + 1 ml 40% MPBS

5. 10^6 cellen B + T 1 ml alle
 + { 2 ml 4% MPBS 6 2 ml 4%
 1 ml PBS 3 1 ml plach
 1 ml laag - $\pm 10^{13}$ Tn → 1-2 x 10¹³

CU Co, 4°C, laagzaam

- Co 5' 1200 rpm
 - ophef in pbs → 50 ml pbs Co 10' 1200 rpm
 - ophef in pbs " "
 + 50 μ l α CO3 - FITC
 + 50 μ l α CO20 - PE
 30' up
 2 x Wassen in buzel
 = ophef in 1 ml PBS - FCS

FACS SORT

cellen in 100 μ l pbs opgevangen: B, T, Eo's, Alles: 1, 10, 100, 1000, 10.000 cellen

- + 150 μ l 76 mM citroenzuur, mengen, 5' RT
 - bij 200 μ l 1M Tris gepipetteerd
 - + 1 ml 2TY
 + 2 ml logendiluent (OD 600 \approx 0.5-0.8)
 30' 37°C
 20' Co 2800 rpm, op bijn laagzaam
 - uitplaten

	1.	10.	100.	1000	10,000	# cells
All cells	1	✓	5	413	640	
B-cells	4	2	220	444	1701	
T-cells	✓	2	1	41	320	
Eos	1	2	9	174	1230	

1.

Kohman's upgarin:	A, B	: all	4
	C, D	: B	6
	E, F	: T	7
	G, H	: Eos	4

2.

All
B
T
Eos

980
6000
3330
390